5-Hydroxymethylation marks a class of neuronal gene regulated by intragenic methylcytosine levels.

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Abstracts

18th Meeting of the Irish Society of Human Genetics, Friday 4th September 2015.

Dublin City University.

PROGRAMME:

10.00 – 10.55	Registration / Tea and Coffee.
10.55 – 11.00	Welcome.
11.00 – 12.15	Oral Presentations. Plenary I: clinical research.
12.15 – 13.15	**Keynote address:** *"The 100,000 Genomes Project"* Prof. Mark Caulfield, William Harvey Research Institute, Bart's and The London School of Medicine and Dentistry, Queen Mary University of London, UK.
13.15 – 14.15	Lunch (Provided) and Poster viewing.
14.00 – 14.15	Council Meeting
14.15 – 15.30	Oral presentations. Plenary II: Basic research.
15.30 – 16.00	Tea and coffee / Poster viewing.
16.00 – 16.15	ISHG AGM.
16.15 – 17.15	**Keynote address**: *"Clinical Implications of 2-day whole genome sequencing of acutely ill infants"* Prof. Stephen Kingsmore, Dee Lyons/ Missouri Endowed Chair in Genomic Medicine, Children's Mercy - Kansas City, USA.
17.15 – 18.00	Wine reception / Presentation of Prizes / Meeting close.

SPOKEN PAPERS:

S01. Diagnostic Yield of the Microarray in Paediatric Practice

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Comparative Genomic Hybridisation (CGH) Microarray has been available in Ireland to Paediatricians from the year 2011. Guidelines for investigation of infants and children with features of developmental delay, dysmorphic features and some cases of epilepsy, recommend the use of CGH Microarray as part of a series of investigations[1].

Our study focused on infants and children screened over the 36 month period from July 2011 to July 2014. Any parent samples sent were excluded and data was entered anonymously on to an excel spreadsheet. Data extracted included; Patient demographics, requesting speciality and indications for testing (Global Developmental delay (GDD), Autism (ASD), Dysmorphic features, Epilepsy and other).

The results showed n=303 children and infants had a microarray sent during the 36 month period chosen. Of these 248 were available for processing. Indications were 46% for Developmental delay, 22.5% for ASD, 14% for dysmorphic features and 12% epilepsy. 23% overall were returned with abnormal Microarray with 14.5% BCNV. Diagnostic yield was 7.3% overall with a 9.5% yield in GDD and 10.9% in ASD. This correlates with previous studies showing a diagnostic yield of 7.8% in GDD and 10.6% in dysmorphic children[2]. In conclusion the Microarray is an investigation in limited selection of disorders and can aid clinicians in obtaining a diagnosis in previously extensively investigated children with no definitive diagnosis.

References: 1. Miller DT *et al*. Consensus statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies. *Am J Hum Genet*. 2010;**86(5):**749. 2. Ellison JW *et al*. Clinical Utility of Chromosomal Microarray Analysis. *Pediatrics* 2012;**130(5)**:e1085-95.

S02. The Next Step in Cardiac Genetics: Targeted gene panels and next generation sequencing in inherited cardiac conditions

G Rea[1,2], JS Ware[1,2], EG Lightman[2], R Walsh[1,2], S John[2] ,T Homfray[3], J Till[3], S Prasad[3], R Buchan[2], S Wilkinson[2], PJR Barton[1,2], SA Cook[1,2,4]

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Inherited cardiac conditions (ICCs) represent a significant cause of morbidity and mortality. Wide variability in expression and penetrance limit the utility of clinical cascade screening in families, whilst the role of molecular diagnosis has traditionally been hampered by the genetic heterogeneity of many ICCs. Currently, new genomic sequencing technologies are transforming the role of molecular genetic testing in ICCs, substantially increasing the diagnostic yield, allowing accurate diagnosis, risk stratification, targeted therapy and cascade molecular screening. We report selected cases from our experience with targeted gene panels coupled with next generation sequencing (NGS) in families with suspected ICC's. Advantages of NGS include the ability to comprehensively sequence large genes such as TTN and RyR2 and the ability to simultaneously sequence multiple genes implicated in disease, including genes for phenocopies, reducing the need to multiple stage testing. We illustrate the impact that targeted gene panels coupled with NGS are having on patient care with respect

to ICCs, using illustrative cases of Dilated Cardiomyopathy, Catecholaminergic Polymorphic Ventricular Tachycardia and Sudden Unexplained Death. The molecular aetiology in these cases would not have been identified using conventional approaches. By demonstrating the transformative potential of this approach we seek to motivate clinicians to move to comprehensive gene panels as a first line for genetic testing in ICCs, to 're - test' previously 'genotype negative' ICC samples and to consider broad based molecular genetic testing in selected novel situations.

S03. An overview of the incidence and impact of MYH polyposis gene mutations in an Irish Cohort

TP McVeigh[1], N Cody[1], M Meany[1], C Carroll[1], R O'Shea[2], DJ Gallagher[2], C Clabby[1], AJ Green[1]

[1]Department of Clinical Genetics, Our Lady's Children's Hospital Crumlin, [2]Mater Misericordiae University Hospital

The MYH gene encodes a DNA-glycosylase enzyme, which is involved in base excision repair. Bi-allelic mutation of MYH confers predisposition to polyposis and gastro-intestinal malignancies, and is distinct genetically and clinically from autosomal dominant adenomatous polyposis coli. In Europe, two common mutations (G382D and Y175C) are reported in 90% of MYH-associated polyposis. We aimed to examine the incidence and impact of MYH mutations in an Irish cohort. A retrospective cohort study was undertaken. Patients tested for MYH mutations were identified by searching electronic patient databases iGene and Crumbase using terms "MUTYH" and "MYH". Patient charts were reviewed for details regarding phenotype and genotype.

Ninety-four patients from forty-one families were tested for MYH mutations. Bi-allelic mutations were identified in eighteen individuals (14 families), and mono-allelic mutations in another 28. At least one of G382D or Y175C was detected in bi-allelic cases. Nine families had bi-allelic status for one/both common European mutations. There was no age difference between mono- and bi-allelic mutation carriers (51±16–v-56±13years, p=0.244). Nine (50%) bi-allelic mutation carriers developed cancer of colon/rectum, compared to 1(4%) patient with mono-allelic mutation. The average age at diagnosis was 49 years (±13). Polyposis was reported in eleven (61%) bi-allelic and 3(11%) mono-allelic mutation carriers.

Mutations were detected in 14/41(34%) families. Bi-allelic MYH mutations confer a strong risk of early-onset colorectal cancer, while risk in mono-allelic carriers reflects that of background population. Screening of bi-allelic mutation carriers is recommended, while screening mono-allelic carriers may not be of any extra benefit over routine national screening programs.

S04. When it comes to exomes, expect the unexpected

J Casey[1], E Crushell[2], J Hughes[2], E Losty[2], D Slattery[3], A Green[4], S Ennis[5], SA Lynch[1]

[1]Genetics Department, Temple Street Children's University Hospital, [2]National Centre for Inherited Metabolic Disorders, Temple Street Children's University Hospital, [3]Respiratory Department, Temple Street Children's University Hospital, [4]Our Lady's Children's Hospital Crumlin, [5]UCD Academic Centre on Rare Diseases.

Consanguinity, or cousin marriage, is widely practised in several global communities. It is well known that consanguinity increases the risk of having a child with an autosomal recessive disorder. Our exome sequencing studies involving consanguineous Irish Traveller families have led to some additional unexpected findings.

Firstly, we found that the risk of incidental findings in consanguineous families is skewed; there is a reduced risk of identifying dominant disorders and carrier status but an increased risk of identifying additional recessive disorders. On average, we analyse <1% of the total exome variants after implementing filtering criteria which should minimise/eliminate the risk of incidental findings. Nevertheless, we have made an incidental finding in 10% of families which is much higher than the expected 3% in non-consanguineous families.

Secondly, we found that 26% of patients who underwent exome sequencing had more than one recessive disorder. In some cases, a second recessive disorder was suspected prior to sequencing. In others, it was unexpected. The presence of two recessive disorders results in unusual phenotypes which complicates making a diagnosis. In most cases, the mutations causing the two recessive disorders are on different chromosomes. However, in some families, the causative mutations are in linkage disequilibrium and co-segregate. It is important to determine whether the two disorders are linked or not as it has important implications for genetic counselling.

In conclusion, our exome studies have raised the interesting observation of patients having multiple recessive disorders, highlighting the need for careful clinical workup and data analysis.

S05. Translating research exome analysis into clinical practice – the Belfast-DDD experience

CW Kirk, S McKee, DDD Project

Northern Ireland Regional Genetics Service

Rare genetic syndromes causing dysmorphism and disability in childhood pose a significant diagnostic challenge, and traditional "one gene at a time" methods result in a diagnostic odyssey that can be time consuming and expensive. The DDD ("Deciphering Developmental Disorders") Study has recruited almost 14,000 cases of rare, presumably genetic, disorders into a pipeline to deliver full exome data as well as copy number analysis, in an effort to identify new disease genes and advance the use of next generation sequencing in diagnostics.

Since the project started in 2012, the NI Regional Genetics Service has recruited over 700 families, making it the second-highest recruiter per capita in the UK. We have received results in 34 cases so far, and analysis is on-going in the others.

Among the genes identified are: ANKRD11, PPP2R1A, PPP2R5D, SATB2, SYNGAP1 and GRIN2B. Many of these diagnoses were unexpected, or the genes unavailable for testing via the routine clinical service.

Feedback from the families has been universally positive, allowing several families to achieve "closure" in relation to the diagnosis in a deceased child, or to appreciate recurrence risks for future pregnancies.

Challenges remain in moving this research pathway into the routine clinical arena, and larger studies such as the UK 100,000 Genomes

Project demonstrate that each new advance is a stepping stone on a long road. We require robust analyses of the clinical and financial benefits of these new technologies, and a clear commitment from health service providers that this is the direction we should be taking.

S06. NRXN1 (Neurexin-1) deletion; A common finding with advent of array but what are its effects?

M Al Shehhi[1], S Shen[2], L Gallagher[3], DR Betts[1], L McArdle[1], A Green[1], SA Lynch[1]

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Recently both point mutations and deletions of neurexin-1 (NRXN1) have been described as a common cause of Autism spectrum disorder (ASD) and other neurodevelopmental disorders. We have identified 37 individuals with NRXN1 deletions in our database of which 22 were index cases. We sought to ascertain the presenting features of index cases and the phenotype, if any, in their relatives.

The cohort was identified by database review of patients referred to our department in whom a neurexin-1 deletion was identified. Chromosome array testing was performed at the cytogenetics laboratory at OLCHC and Guys Hospital London. Genomic data and clinical phenotype information was extracted and analysed.

18/22 (82%) probands were investigated because of developmental delay particularly in speech and language. 4/22 (64%) patients had ASD, 13 had learning difficulties, 4 had seizures. Interestingly, 6/22 were tested because of malformation including congenital heart defect. 8/22 had intronic deletions. 3/22 cases arose de novo. Of the 15 inherited deletions, 2 relatives had a mild ASD phenotype. The majority 13/15 of relatives identified as deletion carriers had a normal phenotype.

Our data suggest that neurexin-1 deletions present with a variable phenotype and are not fully penetrant. Whilst, a parent that carries the deletion has a 50% risk of passing the deletion on, the risk of a child developing ASD is unknown. Genetic Counselling is problematic as the additional factors that trigger the phenotype are unclear. Cascade screening is currently not recommended as it is not possible to predict development of ASD accurately. Intron 5 deletion is pathogenic and further genotype-phenotype correlation studies may help us understand the variability observed.

S07. Transcriptome analysis of CD4+ T cells in coeliac disease

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Department of Clinical Medicine, Trinity College Dublin, St James Hospital, Dublin 8.

Genetic studies have to date identified 43 genome wide significant coeliac disease susceptibility (CD) loci comprising over 70 candidate genes. However, how altered regulation of such disease associated genes contributes to CD pathogenesis remains to be elucidated. Recently there has been considerable emphasis on characterizing cell type specific and stimulus dependent genetic variants. Therefore in this study we used RNA sequencing to profile over 70 transcriptomes of CD4+ T cells, a cell type crucial for CD pathogenesis, in both stimulated and resting samples from individuals with CD and unaffected controls. We identified extensive transcriptional changes across all conditions, with the previously established CD gene IFNy the most strongly up-regulated gene (log2 fold change 4.6; $P_{adjusted} = 2.40x10^{-11}$) in CD4+ T cells from CD patients compared to controls. We show a significant correlation of differentially expressed genes with genetic studies of the disease to date ($P_{adjusted} = 0.002$), and 21 CD candidate susceptibility genes are differentially expressed under one or more of the conditions used in this study. Pathway analysis revealed significant enrichment of immune related processes. Co-expression network analysis identified several modules of coordinately expressed CD genes. Two modules were particularly highly enriched for differentially expressed genes ($P<2.2x10^{-16}$) and highlighted IFNy and other genetically associated transcription factors which showed significantly reduced expression in coeliac samples as key regulatory genes in CD.

S08. Oestrogen withdrawal, breast cell transformation, and breast cancer risk in women with the KRAS-variant

TP McVeigh[1], S Jung[2], D Salzman[3], MJ Kerin[1], S Nallur[3], M Dookwah[3], AA Nemec[3], J Sadofsky[3], T Paranjape[3], O Kelly[3], E. Chan[3], N Miller[1], KJ Sweeney[1], D Zelterman[3], J Sweasy[3], R Pilarski[4], D Telesca[2], JB Weidhaas[2]

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The KRAS-variant rs61764370 is a single nucleotide change in the let7a-binding site of the KRAS gene. It has been previously associated with increased risk of cancer of the breast and ovary. This risk may be modified by environmental factors such as HRT use.

We aimed to evaluate the effect of oestrogen exposure and withdrawal on development of breast cancer in patients with the KRAS-variant.

Isogenic mammary (MCF10A) cell lines with and without KRAS-variant were cultured and observed for oncogenic transformation in charcoal-stripped media following withdrawal and restoration of oestrogen. In vivo investigation was performed by case-control analysis. Data was collected with respect to pathological characteristics, reproductive risk factors and anthropomorphic measurements from a cohort of patients with breast cancer and an unaffected control group of variant carriers.

Addition of tamoxifen to charcoal-stripped media led to 7.9-fold increase in oncogenic transformation in isogenic cell lines with the KRAS-variant, with reduction in colony formation after restitution of oestrogen. In vivo, affected carriers were significantly more likely to have had an oophorectomy pre-diagnosis than wild-type patients ($p=0.033$), and had lower median BMI ($p<0.01$) than unaffected participants with the variant . HRT-discontinuation in variant carriers was significantly associated with post-menopausal triple negative breast cancer ($p<0.0001$), and with cancer of higher grade ($p<0.0001$).

Oestrogen withdrawal in vitro and a low oestrogen state in vivo appear to increase risk of breast cancer and predict aggressive tumour biology in women with the KRAS-variant.

S09. Identifying genetic predictors of skin cancer in renal transplant populations

CP Stapleton[1], M McCormack[1], D Connaughton[2], PJ Phelan[3], GL Cavalleri[1], PJ Conlon[2]

[1]Department of Molecular and Cellular Therapeutics, RCSI, Dublin, [2]Beaumont Hospital Kidney Centre, Dublin, [3]Department of Nephrology, Royal Infirmary of Edinburgh, NHS Lothian.

Renal-transplant recipients have a 33-fold increased risk of developing non-melanoma skin cancer relative to an age-matched non-transplanted individual. In this study we set out to map germline genetic variations influencing the development of skin cancer in our cohort of 325 renal-transplant recipients, using a genome-wide association study (GWAS) and candidate gene study design.

Both logistic regression and survival analysis was applied in our GWAS. Survival analysis was used in our candidate gene study. Multiple robust genetic loci for skin cancer in non-transplant populations have been identified via large GWAS. These genetic predictors of skin cancer (n=21) were examined to see if they have a higher effect size in renal-transplant recipients compared to non-transplant populations.

For the candidate SNP analysis, a nominally significant association was found with a SNP in the MC1R gene (p= 0.0157). The variant was found to have the same direction of affect as described in the original study and the odds ratio was higher. The presence of one or more copies of the minor allele caused a significant decrease in time to developing skin cancer post-renal transplantation (hazard ratio = 2.06). We found a significant association in our GWAS between time to developing skin cancer post transplantation and a variant in SPOCK1 ($p = 4x10^{-8}$). We found that heterozygote individuals developed skin cancer 7 times faster than wild type homozygotes. We will be carrying out further testing in other cohorts for validation of results.

This work is funded by Irish Research Council for Science, Engineering and Technology.

S10. The Irish DNA Atlas– a Study of Genetic Diversity in Ireland

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[1]Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, St Stephen's Green, Dublin 2, Ireland, [2]Genealogical Society of Ireland, 11, Desmond Avenue, Dún Laoghaire, Co. Dublin, Ireland.

Aims: The Irish DNA Atlas is a DNA collection being assembled with the aim of describing the fine-scale population structure in Ireland. Understanding such structure can inform on optimal design of clinical genetic studies as well as the history of the Irish population. We will present an overview of and the preliminary findings from the study.

Methods: We are recruiting individuals with all eight great-grandparents born in Ireland, within 30 kilometres of each other. Participants are asked to complete a detailed birth-brief, which records place and date of birth of three generations of ancestors. We also collect some basic health-related details. DNA is extracted from a saliva sample. We have genotyped using an Illumina OmniExpressdense SNP genotyping platform. We present a number of analyses designed to visualise genetic structure, including; Principle Component, ADMIXTURE, and Runs of Homozygosity analysis.

Results: To date we have recruited 162 participants. The mean great-grandparental area is 32 kilometres, with an average great-grandparental date of birth of 1850. Therefore the individuals in the Atlas provide insight to the genetic landscape of Ireland before significant movement of people from the 20th century onwards. An analysis of dense genotyping data from 142participants shows that the Atlas participants cluster closely with British individuals in a Europe wide PCA, but present different ancestral population components when compared with British, and other European populations. Irish individuals also present slightly higher levels of homozygosity relative to mainland European levels. PCA targeted at specific areas of interest within Ireland also hint at fine-scale substructure.

Conclusion: Ireland shows typical features of a homogenous population, well suited to the study of rare variation in disease risk.

S11. Clinical and genetic predictors of patient response to lacosamide

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There are ~37,000 people in Ireland living with epilepsy. Anti-epileptic drugs (AEDs) control seizures in up to 70% of patients. Lacosamide (LCM) is an AED that is licenced for the treatment of focal-onset seizures. We aimed to determine the clinical and genetic predictors of LCM responsive and non-responsive patients. A total of 483 patients, who were previously refractory to medication, were recruited from four tertiary epilepsy referral centres: Dublin, Ireland; London, UK; Brussels, Belgium; North Carolina, USA. Response to LCM was determined according to four categories; (i) seizure freedom, (ii) ≥75% reduction in seizure frequency, (iii) seizures worsening and (iv) no response. Overall, 13% of patients showed a positive response (seizure freedom or ≥75% reduction in seizure frequency) to LCM treatment. Response varied depending on epilepsy diagnosis, with idiopathic generalised epilepsy (also known as genetic generalised epilepsy) emerging as a potential target group for LCM treatment. An adverse drug reaction causing discontinuation of LCM treatment was recorded in 19% of patients. Genome wide association (GWAS) and whole exome sequencing (WES) was used to investigate the importance of genetic variation in predicting LCM response. Analysis of common variation via GWAS pointed to a locus containing the KALRN gene as potentially predictive of membership to the seizures worsening group. Analysis of rare variation via WES did not identify any additional variant or gene associated with particular LCM response groups.

S12. Unfolded Protein Response in a mouse model of Messmann's Epithelial Corneal Dystrophy

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Corneal dystrophies are a group of blinding inheritable conditions with a collective worldwide incidence of 1 in 2,000. Meesmann's epithelial corneal dystrophy (MECD) is a rare autosomal dominant disorder with phenotypes of varying severity ranging from asymptomatic to foreign body sensation, photophobia, presence of anterior epithelium microcysts and corneal scarring. It is caused by dominant-negative heterozygous missense mutations found within the KRT3 or KRT12 genes encoding the cytoskeletal keratins K3 and K12. To investigate the pathomechanism of this disease we generated and characterized a novel knock-in humanised mouse model carrying the MECD-associated Leu132Pro mutation.

Although no overt changes in corneal opacity were detected by slit-lamp examination, heterozygous mice exhibited a subtle histological and ultrastructural phenotype of cell fragility within the corneal epithelium, which was greatly exaggerated in homozygous animals. Mutant corneal epithelial cells were larger, contained prominent intracellular spaces and showed overt cytolysis with occasional cell rupture at the corneal surface.

Immunohistochemical analysis showed that the humanized mutant K12 protein was expressed specifically in the anterior corneal epithelium and revealed an altered keratin expression profile in the cornea of mutant mice that was confirmed by quantitative Western blot analysis.

Analysis of expression of unfolded protein response (UPR) markers, Caspase 12 and DDIT3, revealed up regulation of both markers in homozygous mice and DDIT3 in the heterozygous mice. A TUNEL assay revealed that the apoptotic rate in the mutant cornea was increased 17–fold compared to the wild type ($p<0.001$).

Thus, we have developed a novel mouse model for MECD, which up-regulates UPR pathways that will be a valuable resource for development of therapeutics targeting dominant-negative corneal dystrophies.

POSTER PRESENTATIONS:

P01. A Neurofibromatosis type 1 database for ROI

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Neurofibromatosis type 1 (NF1) is a multisystem variable genetic condition that causes benign tumours in various organs, mainly the skin, eyes and brain. Other associated health complications are epilepsy, scoliosis, learning difficulties and autism. Our aim was to set up a database of the NF1 patients who had been through our Service since it was set up in 1995, detailing how they were affected by the condition. We run a fortnightly NF clinic that is funded by NF Ireland. We used the database template that is used in the Belfast NF clinic.

A cohort of 575 patients with NF1 was recorded on the database on 20/02/2015. The prevalence of NF1 is reported to be 1/3000 live births and based on the estimated population there should be 1,600 individuals with NF1 in ROI.

We identified 13 women with NF1 who were in their 40's and warranted breast surveillance. With their permission, we wrote to their GPs requesting that this be put in place.

We also identified 24 individuals who would be transitioning from Paediatric to Adult services. We wrote to them offering a Genetics Consultation to discuss screening for adults with NF1 and the genetics of the condition. So far, 5 have accepted this offer.

Already this database is proving to be a useful tool for monitoring and identifying NF1 patients who may benefit from screening and/or Genetics review. Also, recording the clinical features of NF1 patients will help us to identify those patients with complex NF1 who require Specialist input.

P02. Titin Truncating Variants are common in patients with myocardial infarction and low ejection fraction

G Rea [1,2], J Petyrka[3], M Vieira[3] ,JS Ware[1,2], R Walsh[1,2], S John [2], S Prasad[3], R Buchan[2], S Wilkinson[2], S Prasad[2], PJR Barton[1,2], SA Cook[1,2,3]

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The Titin gene is a major determinant of myocardial function and its importance in familial and 'idiopathic' Dilated Cardiomyopathy (DCM) has recently been ascertained. We hypothesize that patients with pronounced left ventricular dysfunction following myocardial infarction (MI), when controlling for infarct parameters and coronary anatomy, may have a high burden of TTN truncating variants (TTNtvs). We studied a large cohort (n=335) of post-MI patients. Gadolinium-enhanced Cardiac Magnetic Resonance (CMR) was used to characterise cardiac dimensions, function and tissue properties, the size and thickness of MI were quantified using a standard 17-segment model. Targeted re-sequencing of TTN was performed. Genetic variation in TTN in 430 ethnically matched healthy volunteers along with public repositories, were used for variant annotation and comparison. Our analyses show that out of the 335 post-MI patients, nine (2.7%; ~1 in 35 post-MI cases) had a TTNtv. Patients with a TTNtv had a significantly lower LVEF than those without (31.2±13.9% vs. 41.2±14.7%; p=0.026). An LVEF <30% is used to guide device therapy in post-MI patients and as hypothesized, TTNtv were significantly enriched in this group compared to MI patients with higher LVEFs (6.98% vs. 1.21%, p=0.01). These data identify a novel role for truncating variants in TTN, a DCM gene, in post-MI systolic dysfunction. Intriguingly, the effect size of the TTNtv is equal or greater than many of the infarct covariates used to guide re-vascularisation therapy. Based on these findings it will be important to explore if genetic stratification of the post-MI patient can inform treatment strategies.

P03. The extent of Lymphangioleiomyomatosis (LAM) in the Tuberous Sclerosis (TS) population in NI

DE Donnelly[1], T O'Neill[1], R Hardy[1], PJ Morrison[1]

Belfast Health & Social Care Trust, Belfast.

Lymphangioleiomyomatosis (LAM) is a multisystem disease affecting, almost exclusively female, TS patients. It is characterised by cystic lung destruction and symptoms occur from the 2nd or 3rd decade onwards. Recently, new treatments, e.g. mammalian target of rapamycin (mTOR) inhibitors, have been found to be effective. It is important that women are screened for symptoms for this condition to allow for early intervention. We have 43 women with TS over the age of 18 years. All were sent out a questionnaire regarding respiratory symptoms, treatment and follow-up to determine the extent of LAM in our population. We aim to use this data to improve screening for LAM in our regional TS clinic.

P04. Connexin 26/30 & deafness: our experience of 5 years

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[1]Queen's University, Belfast, [2]Medical Genetics Department, Belfast City Hospital, BT9 7AB

Congenital hearing loss is the most prevalent and genetically heterogenous sensorineural disorder. Around 50% congenital hearing loss in genetic in nature and can be syndromic (30%) or non syndromic (70 %). Autosomal recessive non syndromic deafness is the commonest cause of genetic hearing loss predominantly related to connexin 26/30 genes. The genetic forms of hearing loss are diagnosed by otologic, audiologic, and physical examination, family history, ancillary testing (e.g. temporal bone CT, ECG), and molecular genetic testing. In the absence of a specific diagnosis, empiric recurrence risk coupled with connexin 26/30 results is used for genetic counselling. We analysed our data of past 5 years (2010-14) of connexin 26/30 gene mutation analysis. Connexin 26/31 gene mutation analysis was done in 61 individuals. Fifteen had developmental delay and/or dysmorphism and eighteen had other genetic investigations either pre or post connexin 26/30 testing. Temporal bone imagining and ECG was not done routinely .Three had array CGH identified chromosomal abnormality, two had confirmed diagnosis of Pendred syndrome and three were investigated for Waardenburg syndrome. Connexin 26/30 mutation related deafness was identified in 8 individuals (13%). Five were homozygous for 35delG and three were compound heterozygous. All the mutation positive individuals had bilateral, prelingual, severe to profound SNHL with no dysmorphism or developmental delay excluding the speech. The antenatal and postnatal history was not significant and the inner ear imaging was normal. When these characteristics were taken into consideration the diagnostic yield improved to 22 % highlighting the phenotype associated with Connexin 26/30 related hearing loss.

P05. Initial experience on transportation based PGD/PGS in Ireland

X Zhang[1], T Dineen[1], J Flanagan[1], A Kovacs[1], R Mihart[1], J O'Callaghan[1], J Culligan[1], N Daly[1], D McAuliffe[1], J Waterstone[1]

Preimplantation genetic diagnosis/screening (PGD)/ (PGS) is a reproductive option for couples at risk of a genetically abnormal pregnancy. This study reviews the outcome of all biopsy cycles performed at Cork Fertility Centre (CFC) since the programme began.

Embryo biopsy and embryo transfer procedures were performed at CFC while genetic analysis of the biopsy cells was carried out at Reprogenetics, UK. Biopsied embryos were cryopreserved by vitrification. Unaffected embryos were warmed and transferred in a subsequent frozen embryo transfer (FET) cycle. Seven couples have completed a PGD/PGS cycle at CFC, five for single gene disorders, (cystic fibrosis-3 cases, Mucolipidosis and Smith-Lemli-Opitz Syndrome). The other two couples underwent PGS.

In total, 39 embryos were biopsied, 15 on day 3 (one cycle) and 24 on day 5/6.No logistic failures were found during the biopsy sample transportation. Genetic defects were detected using multiplex-PCR, karyomapping, and array CGH. Polymorphisms were used to confirm diagnosis, and detect chromosome abnormalities. Genetic testing identified 15 unaffected and 19 affected embryos with single gene disorders. 12 blastocysts (54.5%) were chromosomally euploid.

Seven out of eight unaffected embryos survived after warming and were transferred in 6 FET cycles. Four pregnancies resulted -two live births, two ongoing pregnancies- an implantation rate of 57%.

Combining blastocyst biopsy with vitrification appears to be an effective strategy for transportation PGD/PGS. The on-going pregnancy rate suggests that a transport PGD/PGS programme can be an effective reproductive option for couples seeking genetic testing of embryos prior to implantation.

P06. Investigating the role of a single nucleotide polymorphism at 9q22.23 in Thyroid Cancer Predisposition: A Case-Control Study

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FOXE1 is an intronless gene located on chromosome 9q22.23. FOXE1 plays a crucial role in thyroid morphogenesis. Mutations in FOXE1 are associated with a number of thyroid pathologies, namely hypothyroidism, athyroidism and thyroid cancer.

This study aims to investigate the frequency and impact of a single nucleotide polymorphism G>A at 9q22.23 in a Western European cohort of patients with thyroid cancer compared to controls.

DNA was extracted from buccal swabs or whole blood of patients with differentiated non-medullary thyroid cancer by ethanol precipitation. Patients were recruited from two tertiary referral centres in Ireland and France. Cancer-free controls were recruited from the community. Genotyping was performed using Taqman-based PCR. Data was analysed using SPSS V22.

One hundred and eighty one cases and eighty-three controls were genotyped for the variant. The frequency of the minor allele among cases was 0.46 compared to 0.30 among controls. Genotypic frequencies and odds ratios are outlined in the table. The variant was identified in patients with thyroid cancer significantly more frequently than controls in both heterozygous and homozygous forms. This supports the role of this variant in thyroid predisposition.

	Common Homozygote	Heterozygote	Rare Homozygote
Control	41	34	8
Case	49	98	34
Odds Ratio	-	2.41 (1.36-4.26)	3.56 (1.48-8.53)
	-	0.002	0.003

P07. A qualitative analysis of the attitudes of Irish patients towards participation in genetic-based research

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Background: Progress in diagnostic and therapeutic strategies in medicine is dependent upon high-quality biomedical research. Translation of research findings into the clinic relies on patient participation in innovative clinical trials. We investigated attitudes to genetic research in Ireland, in particular with respect to commercial and financial implications.

Methods: A multi-centre cross-sectional survey study was performed. Consecutive patients attending four out-patient clinics were asked to complete paper-based questionnaires. An electronic version of the same questionnaire was created on Survey Monkey with a link made public on a social media website for a period of 24 hours. Data was analysed using SPSS.

Results: 351 questionnaires were completed (99 paper, 252 electronic). The majority of respondents were female (n=288, 82%), and highly educated, with 244 (70%) attending college/university. Most participants supported genetic research (267, 76%), more frequently for common diseases (274, 78%) than rare disorders (204, 58%, $p<0.001$, x2). 103 (29%) had participated in scientific research, and 57(16%) had donated material to a bio-bank. The majority (n=213, 61%) would not support research with potential financial/commercial gain. 106(30%) would decline to participate in research if researchers would benefit financially, compared to 49(14%) if the research was supported by a pharmaceutical company, ($p<0.001$, x2). Respondents would provide buccal samples (258, 74%) more readily than tissue (225, 64%) or blood (222, 63%).

Conclusion: A high level of support for genetic research exists among the Irish population, but active participation is dependent upon a number of factors, notably, type of biological material required, frequency of the disease in question, and commercial interest of the researchers.

P08. Pedigree Drawing in the Department of Clinical Genetics: An Audit of Adherence to International Recommendations

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A pedigree is the symbolic language of clinical genetic services and a visual representation of a family's medical history and genetic relationships. It assists diagnosis, identification of relatives at risk, and is a crucial tool in identifying inheritance patterns of genetic disorders. We aimed to examine adherence of clinical genetics professionals of the Department of Clinical Genetics (DCG) to international guidelines for pedigree drawing.

A retrospective chart review of pedigrees drawn in 102 consecutive outpatient appointments in October 2014 was undertaken. Each pedigree was scored using a standardised proforma adapted from international guidelines. Data was recorded with respect to referral type, legibility and pedigree complexity.

Pedigrees were completed in 98(96%) charts. The median score obtained was 10/20(2-16). Pedigree identifier was recorded in 30(31%). Sixty-one (62%) pedigrees were signed, and 57(58%) dated. The proband was identified in 34(35%), with date of birth (DOB) recorded in 32(32%). First degree relatives (FDRs) DOBs were stated for 24(25%), and age only in 31 (32%). Ethnicity was recorded in 13(13%). Presence/absence of consanguinity was noted in 24 (24%). The majority of pedigrees included ≥3 generations 94 (96%), including a median of 22 (3-66) individuals.

Although the majority of pedigrees contained large volumes of data on multiple individuals across multiple generations, deficiencies were identified in specific areas. While pedigree drawing should be a standard competency for all healthcare professionals, genetic professionals' standards should be exemplary. Results were presented at a Departmental Clinical Meeting, impediments to adherence with guidelines identified and recommendations for improvement discussed. Re-audit is currently ongoing.

P09. Beware of the genome

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Exome and genome sequencing are being hailed as important tools in diagnostics and personalised medicine. This technology has already integrated into some aspects of mainstream medicine in Ireland. But are we overlooking the scale of the challenges and pitfalls that these tests present? Here, we discuss a number of issues that we have encountered during our research studies.

Incidentals and VOUS: The European Society of Human Genetics advocates for stringent filtering of data to minimise the risk of incidental findings. However, our research shows that incidental findings can still occur, despite using stringent filters. Counselling can be difficult even in cases where the incidentals are of proven pathogenicity. Furthermore, a large proportion of incidentals are variants of unknown significance (VOUS), making interpretation and reporting very challenging. Should we act on VOUS? And at what cost to the healthcare system?

Misclassified variants: Our understanding of how genetic variants impact our health is still in progress. It is not surprising that recent studies are showing that variants originally reported as pathogenic are actually benign. As new information emerges, it is possible that variant classification will change. What are the implications for patient counselling?

Filtering: There are numerous tools and databases available for data filtering. It is now becoming evident that different analysis tools

give different results. The lack of standardisation could pose a significant problem, particularly in a clinical setting?

Whilst exome/genome sequencing has significant potential to improve diagnostic yield, it is important that all stakeholders are aware of the potential pitfalls and challenges to ensure safe implementation of this new technology.

P10. YouTube, animation and genetic education

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We have developed a number of short videos, some animated, to help develop integrated online genetic education. Our vision is to develop core videos on topics such as pedigree drawing, genetic cascade and predictive testing, exome/genome sequencing, incidental findings and ethics, relevant to practises in Ireland. Outside of the core, we have specialist topic videos relevant to genetics, created by colleagues from diverse disciplines. Our target audience are health care professionals from all aspects of mainstream medicine.

Much of our content, such as our six animated videos, are freely available on YouTube. These videos already have ~30,000 views. Families can view them prior to an appointment.

We found that short videos (~5 minutes) are more popular; the viewer stays with the video through its entirety in contrast to long videos. As viewers post thumbs up and thumbs down as well as comments, this is a useful way of gaining prompt feedback. Our two recent chromosome translocation videos have already had >18,000 views and links to the ESHG website have been provided. We are translating these videos into ten languages to increase applicability. http://bit.ly/RecipTranslocation & http://bit.ly/RobsTranslocation

Feedback includes: "Wow this video was more helpful than any other genetic video on YouTube" and "Now I understand it thanks".

Whilst YouTube is used by the public to access genetic information, much of the educational content is aimed as researchers. There is a market for simple genetic information to be developed for the public.

Grants: UCD; Temple Street children's fund for Health; Shire Pharmaceuticals

P11. A Case of Metaphyseal Chondromatosis with D-2 Hydroxyglutaric Aciduria

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A developmentally appropriate thirteen month old girl, born to non-consanguineous Irish parents, presented with asymmetry or an extra crease in the midshaft of her right arm and relative short stature (occipitofrontal circumference - 91st centile, weight - 50th-75th centile, Length - 0.4th-2nd centile). A radiograph of the arm followed by a skeletal survey revealed bilateral symmetrical irregularities of the metaphyses of the humeri (proximal), femora (proximal and distal), and tibiae (proximal and distal) with less marked changes in the fibulae, feet and phalanges. Irregular chondral dysplastic changes in the left iliac blade were also noted. The epiphyses were spared, the skull, vertebrae, ribs, clavicles were unremarkable and bone age was normal. Urinary D2-Hydroxyglutarate was approximately 55 times the normal level confirming a diagnosis of metaphyseal chondromatosis with D-2 hydroxyglutaric aciduria. This is likely due to somatic mutations in the isocitrate dehydrogenase gene which is currently under analysis. The prognosis is guarded and the recurrence risk is considered to be <1%. This case highlights 1: an unusual combination of characteristic skeletal and metabolic abnormalities which has rarely been reported and 2: the importance of performing urine organic acid in patients who present with generalized enchondromatosis.

P12. Segmental overgrowth syndromes caused by somatic mosaic mutations in PIK3CA

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Patient Age and Sex	Part of Body Affected with Overgrowth	Learning Disability	Other findings	Gene Mutation
2 year old boy	Left Leg (particularly 4th and 5th toes)	No	No	PIK3CA (H1047L) Left leg (10-20 % cells) Right leg (0% cells)
16 year old girl	Generally Overgrown, Right hemihypertrophy	Mild	Capillary Malformation	PIK3CA (E418K) Right arm (50% cells) Left arm (25% cells)
6 week old girl	Right Foot	No	Displaced bones in foot	Result awaited
13 month old girl	Macrocephaly	No	Polymicrogyria	Result awaited
16 year old boy	Generally overgrown, left side larger than right below neck, opposite above neck	Mild	Capillary Malformation	Result awaited
3 year old boy	Hemimegalencaphaly	Mild/Moderate	Naevus flammeus	Result awaited
11 month old girl	Right leg	No	Right Hydronephrosis	Result awaited

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Segmental overgrowth syndromes are rare, poorly classified disorders which carry a significant burden of morbidity and mortality that pose diagnostic, prognostic and management challenges. Recently, somatic mutations in the phosphatidylinositol-3-kinase/AKT/mTOR [PROS] cellular signalling pathway have been shown to underline many overgrowth disorders. We present 7 cases of segmental overgrowth presenting with wide phenotypic variation (see Table). Pathogenic somatic mutations in PIK3CA were identified in two cases to date. Genetic analysis is awaited on the other four cases. Such cases are reclassifying segmental overgrowth disorders and helping the development of targeted therapies such as mTOR inhibitors. A collaboration with Cambridge University is facilitating a further 35 cases of segmental overgrowth to be enrolled for investigation and possibly inclusion in a treatment clinical trial.

P13. Microdeletion/microduplication of the proximal 15q11.2 (BP1-BP3) region: an emerging susceptibility locus

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The proximal long arm of chromosome 15 contains clusters of LCRs located at five common breakpoint sites, referred to as BP1-BP5. This region is susceptible to rearrangements mediated by non-allelic homologous recombination which results in various deletions and duplications. Two common classes of deletions are described in individuals with Prader-Willi /Angelman syndrome (PWS/AS). PWS/AS deletion is flanked by either proximal BP1 or BP2 and the more distal BP3. Individuals with PWS/AS with Type I deletions (BP1-BP3) have been reported with more severe phenotype than individuals with Type II deletions (BP2-BP3). The BP1-BP2 region spans approximately 500kb and contains four highly conserved genes, TUBGCP5, NIPA1, NIPA2 and CYFIP1; TUBGCP5 is expressed in subthalamic nuclei while the latter three genes are widely expressed in the central nervous system. Recent studies have suggested an association between BP1-BP2 deletions/duplications with an abnormal clinical phenotype including dysmorphisms, speech and motor delay, autism, behavioural problems and seizures. However, imbalances in this region have also been seen in the normal population and in mildly affected carriers suggesting that the region contains genetic material associated with incomplete penetrance. We identified 27 patients with imbalances within the proximal 15q11.2 (BP1-BP2) region, all presenting with various degrees of developmental delay. Analysis was conducted using a high resolution microarray-based comparative genomic hybridization (aCGH). Parental studies were carried out where possible. The results presented here indicate that 15q11.2 BP1-BP2 copy number changes may increase susceptibility to neurodevelopmental problems.

P14. Orphanet Ireland : Mapping Ireland's Rare Disease Activity

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Orphanet is an international information portal for rare disease (RD) activity. 39 countries participate in rare diseases data collection, with over 41,000 daily site hits to www.orpha.net from over 200 countries. Disease summaries are written by experts and link to more detailed information. Data collected includes clinical expert centres, medical laboratories, patient organizations, research projects, registries, clinical trials and biobanks; as well as reports on RD prevalence and orphan drugs.

Orphanet Ireland is funded by the EC 3rd Joint action on RD and the HSE, and located at the National Rare Diseases Office. Our short term goal is to ensure the accuracy of the existing Irish data, then to create a comprehensive rare disease resource database within the next 2 years.

Data on all RD activity can be self-declared by clinicians, researchers and patient organizations but is verified and validated by the Orphanet Ireland to ensure it meets inclusion criteria. Data will also be collected from departmental and professional websites, regulatory and funding bodies, as well as direct contact with professionals.

The aim of centralizing Irish rare disease information is 1: to provide a resource to clinicians and patients looking for or with a new RD diagnosis, and 2: to promote links between professionals and the patients concerned, within and between countries. Orphanet Ireland will serve as the unifying platform for RD activity for designation of centres of expertise (ongoing) and of laboratories and researchers participating in Reference Networks (from 2016) to fulfil Ireland's European and Cross Border Directive requirements.

P15. miR-24 regulates p27 expression in prostate cancer

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MicroRNAs (miRNAs) are small, non-coding RNA molecules with an important role in cancer. In prostate cancer, several miRNAs are expressed abnormally suggesting they may be useful markers for diagnosis, prognosis, and potential therapeutic intervention in this disease. In this study we used PCR to investigate the expression of miR-24 in a panel of prostate cancer cell-lines and in a series of clinical prostate biopsy specimens. The biological significance of miR-24 expression in prostate cancer cells was assessed by a series of in vitro bioassays and the effect on proposed targets p27 (CDKN1B) and p16 (CDK2NA) was investigated. We showed that miR-24 expression was significantly lower in prostate cancer cell lines compared to a normal prostate epithelial cell line. Decreased expression of miR-24 was also more frequently observed in both needle core and prostatectomy tumour tissue relative to matched normal tissue. Low miR-24 expression correlated with high PSA serum levels and other markers of increased prostate cancer progression. Importantly, over-expression of miR-24 inhibited cell cycle, proliferation, migration and clonogenic potential of prostate cancer cells, as well as inducing apoptosis. p27 and p16

were confirmed as targets of miR-24 in prostate cancer cells and a significant inverse correlation between miR-24 and p27 was revealed in clinical prostatectomy specimens. These findings provide evidence that miR-24 has a tumour suppressor role in prostate cancer and also targets p27 and p16 in prostate cancer cells. We propose that it may be a useful progression biomarker or focus of therapeutic intervention for this disease.

P16. Cognitive analysis of schizophrenia risk genes: focus on genes with epigenetic function

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Schizophrenia is a psychiatric disorder characterised by positive and negative symptoms as well as cognitive impairment. Disrupted epigenetic processes are observed in complex and single gene brain disorders that exhibit cognitive deficits, and have been recently studied as potential targets of pharmaceutical intervention for the treatment of cognitive deficits.

Genome wide association studies (GWAS) have identified 108 chromosomal regions associated with risk of schizophrenia, implicating 350 genes. The aim of this study was to identify risk genes for schizophrenia with epigenetic functions and test these genes for association with cognitive deficits in schizophrenia. Cross-referencing 535 epigenetic genes with 350 GWAS genes identified 5 candidate genes: RERE, SATB2, EPC2, EP300 and KDM3B. The effect of risk single nucleotide polymorphisms (SNPs) in these genes on cognition was examined using a dataset of psychosis cases (n = 905) and controls (n = 330) who had completed tests in 5 areas of cognition: IQ, working & episodic memory, attention and social cognition. Regression was carried out using a linear model. For RERE, there was association between the schizophrenia risk allele and attention ($p = 0.03$). For SATB2, there was association with social cognition ($p = 0.003$). For EPC2, an association was found with full scale IQ ($p = 0.004$) and performance IQ ($p = 0.001$). An association was found between the schizophrenia risk allele for KDM3B and verbal IQ ($p = 0.038$). This initial analysis provides support for our hypothesis that risk genes with epigenetic functions contribute to cognitive deficits in schizophrenia.

P17. Do Irish periodic paralysis patients have a common genetic origin?

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Periodic Paralyses (PPs) are rare autosomal dominantly inherited skeletal muscle channelopathies characterised by episodic weakness secondary to abnormal muscle excitability. PPs are broadly classified into hyperkalaemic (HyperPP) or hypokalaemic (HypoPP) based on serum potassium (K^+) levels. HypoPP is caused by mutations in CACNA1S and SCN4A awhile the less frequent HyperPP is generally caused by SCN4A gene mutations.

We have recruited a growing cohort of Irish PP patients for which comprehensive clinical and genetic data has been gathered. We believe that this group of PP patients are phenotypically and genetically distinct. Firstly, contrary to publications, we have detected more HyperPP than HypoPP. Secondly, we have detected only one of the common SCN4A gene mutations and finally each of the patients is unusual clinically with attacks of longer duration and increased severity.

The aim of this project is to investigate this group of Irish periodic paralysis patients in which the same gene defect has been described to determine whether there is a common genetic background.

We will use haplotype analysis of polymorphic microsatellite markers in the SCN4A gene region to determine if there is a shared genomic region.

We will present the results of this study and discuss the implications for the diagnosis and management of these patients.

P18. PCR-RFLP assay for the detection of LHON Mutations

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Leber hereditary optic neuropathy (LHON) is one of the most common inherited optic neuropathies with an incidence of up to 1 in 31,000 worldwide and results in significant visual morbidity among young adults. The disorder is the result of mitochondrial dysfunction and results from primary mitochondrial DNA mutations affecting complex I subunits of the respiratory chain. Approximately 95% of LHON patients will have one of 3 mitochondrial mutations, G3460A (13%), G11778A (69%) and T14484C (14%) in NADH Dehydrogenase subunits 1, 4 and 6 respectively with other rare mutations accounting for the final 5%. Visual recovery can occur in some LHON patients but the extent of the visual recovery is influenced by the mutation involved, highlighting the need for a simple robust and cost effective mutation detection strategy.

The 3 common mutations are typically identified by individual end-point PCR-RFLP, ARMS PCR or PCR followed by Sanger / Pyrosequencing. This study developed a multiplex PCR-RFLP assay to detect the 3 common LHON causing mutations in a single tube format.

Primers, based on the reference sequence NC_012920.1, were designed to incorporate a MaeIII restriction site in the presence of the 3460A, 11778A and 14484C mutations and the multiplex assay reliably detected the 3 mutations in LHON patient DNA and in synthetic LHON controls harbouring the 3 common mutations cloned into plasmids.

In conclusion, we developed a simple cost effective assay to detect 95% of LHON causing mutations and developed a set of cloned controls providing an unlimited patient free resource for LHON testing.

P19. Targeting hypoxia in prostate cancer cells to increase treatment efficacy

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Androgen deprivation therapy e.g. bicalutamide (BCA) is widely used to treat advanced prostate cancer; however, within 1.5-3 years most tumours have progressed to androgen independence (Abate-Shen C; Genes Dev 14, 2410-2434; 2000). Previously we showed that daily BCA causes an initial profound hypoxia (<0.1%) in LNCaP tumours that recovered after ~17 days. This was accompanied by progression to a more malignant phenotype indicating hypoxia-driven treatment failure (Ming L. *Int J Cancer* 2013;**132:**1323-1332). Combination with AQ4N, a unidirectional hypoxia activated pro-drug (uHAP), blocked this progression. We have now characterised gene expression changes during treatment and have investigated the effect of a novel uHAP (OCT1002) in blocking these effects. LNCaP prostospheres were treated with vehicle, BCA, OCT1002 and combination therapy. Prostosphere size was measured pre and post treatment. Clonogenic assays and flow cytometry was carried out on treated prostospheres to measure changes in invasive potential and apoptosis, respectively. Furthermore, LNCaP-Luciferase expressing cells ($4x10^6$) were implanted into SCID mice, treatment (as above) commenced when tumours reached ~$150mm^3$. Tumours were measured every 2 days using calipers. Bioluminescence in the lung was calculated at the experimental endpoint. In vitro and in vivo data reveal the potential of OCT1002 when used in combination with BCA. Combination treated prostospheres had stunned growth rate, higher apoptosis and reduced colony formation compared to control groups. In vivo data shows that combination therapy results in a significant tumour growth delay and reduced lung metastases by approximately 40%.

P20. Schizophrenia-associated SNPs proximal to neurotransmission genes impact cognitive performance in patients and controls

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Schizophrenia (SZ) is characterised by positive, negative and cognitive symptoms. As SZ is highly heritable, recent research has focused on GWAS, the most recent of which identified 83 new regions of interest associated with SZ. The link between these and specific functions in SZ is currently unknown. To take these results forward, these need to be identified, be that at the level of protein, neural pathway or phenotype.

To characterise the effect of SNPs on cognitive function, first a selection was carried out based on the following classifications 1: proximity of SNP to gene, 2: unique association of this gene to SNP, and 3: gene involvement in neurotransmission, which is disrupted in SZ. This resulted in a selection of eleven SNPs in close proximity to ten genes: four involved in glutamatergic neurotransmission (GRM3, GRIN2A, SRR, CLCN3), five signalling (CACNA1C, CACNB2, HCN1, RIMS1) and two receptor genes (DRD2, CHRN). Neuropsychological measures of social cognition (Reading the Mind in the Eyes, Hinting Task, Internal, Personal, Situational and Attributional Questionnaire) were analysed to assess the impact of each SNP on social cognitive function.

Analyses indicated a significant effect on measures of cognition in patients and controls. SNPs in the regions of CACNB2 (r^2=0.032, p=0.001) and RIMS1 (r^2=0.007, p=0.032) show a significant effect on scores on the Hinting Task and Reading the Mind in the Eyes, respectively. These findings implicate risk SNP effects on the generalised neurotransmission process as opposed to any one specific neurotransmitter.

P21. Impaired cognition in schizophrenia: Genetic risk factors related to MHC loci

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Introduction: Although the etiology of schizophrenia (SZ) is largely unknown, it is increasingly clear that genetic and environmental interactions contribute to cognitive deficits associated with this disorder. Recent Genome wide association studies (GWAS) have indicated a link between SZ and immune dysregulation, especially genetic mutations related to the major histocompatibility complex (MHC). Cognitive deficits are core features of Schizophrenia and related disorders, which relate to genetic risk. This study aims to explore the relationship between MHC risk variants for SZ and cognitive deficits, while also relating findings to brain activity.

Methods: To test if MHC risk variants impair cognition, ANCOVA analysis is performed on genetics data previously collected in a GWAS. Cognition measures are compared in groups with and without MHC genetic risk, in a population of SZ sufferers and healthy controls. Functional MRI imaging will also be performed to test if genetic risk relates to altered neural activity.

Results: Preliminary analyses suggest that MHC risk variants contribute to impairments in cognition in domains of social cognition, IQ and attention. Further analysis will be performed to test for environmental mediators of this relationship, looking at cannabis use and urbanicity. BOLD fMRI will also be used to test for a relationship between MHC risk and altered neural activity, using MATLAB SPM.

Conclusions: The MHC genetic variant may serve as a significant risk marker for schizophrenia, and further elucidate etiology of this neurodevelopmental disorder. Future studies on neurobiology of social cognition, and greater knowledge of genetic risk may establish targets for interventions.

P22. In vivo gene silencing by siRNA delivery to the corneal epithelium in a keratin-12- bioluminescence mouse model

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Aim: To create a bioluminescence mouse model which expresses firefly luciferase in the corneal epithelium to assess gene editing and gene silencing for the cornea.

Methods: A gene targeting vector was generated where the Krt12 coding sequence in and the splice donor site of exon 1 were replaced with a transgene cassette containing a luc2-Multiple Targeting Cassette (MTC) gene fusion. The vector was transfected by electroporation into the Taconic Artemis C57BL/6N Tac ES cell line. Homologous recombinant clones were isolated and validated, and the mice bred with luc2-positive/ PuroR-negative offspring used for colony establishment.

To visualise the expression of luc2 within the corneal epithelium, luciferin substrate diluted in viscotears was applied to the front of the eye and then luciferase expression was imaged and assessed using a Xenogen IVIS Lumina Imager and LivingImage 3.2 software.

Intrastromal injection of siGlo siRNA was used to determine the localisation of siRNA within the corneal epithelium and then the established mouse model was treated with either native or Accell "self-delivery" siRNA.

Results: The Accell "self-delivery" siRNA induced potent sustained allele specific silencing for 7 days, while native versions of siRNA resulted in significant knock-down for 1 day only ($p<0.05$).

We have created and validated a bioluminescence mouse model and have utilised it to assess siRNA in vivo. This mouse model coupled with the Lumina imager will allow us to assess topical delivery of gene therapies to the ocular surface allowing validation for future translation to clinical use.

P23. Mutant allele-specific gene silencing in autosomal dominantly inherited Fuchs' corneal dystrophy using CRISPR Cas9 nuclease

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Dominant-negative or gain-of-function disease-causing mutations are not suitable for gene supplementation therapies. The CRISPR Cas9 system has emerged as a powerful in vivo and in vitro RNA-guided sequence specific nuclease. Critical for target site recognition by the Cas9 nuclease, a 3bp protospacer-adjacent motif (PAM) is located adjacent to its 3'end. A review of known point mutations that cause corneal dystrophy showed that over 30% result in the formation of a novel PAM site. To investigate whether these mutations could be specifically targeted by CRISPR/ Cas9 we focused our attention on the Fuchs' endothelial corneal dystrophy (FECD), where the L450W mutation in the alpha2 chain of Collagen VIII (COL8A2) gene creates a new PAM site absent in the wild-type allele.

In vitro assays were designed to determine the efficiency and specificity of Cas9 cleavage. Wild-type and mutant COL8A2 genes, cloned into a Luciferase reporter vector, together with single guide RNA (sgRNA) Cas9 constructs, were used to transfect AD293 cells. The sgRNA targeting Cas9 to a site adjacent to the L450W mutation showed a significant ($50\% \pm 3.9\%$, $p<0.01$) knockdown of mutant allele expression, without effect on wild type.

AD293 cells were co-transfected with equimolar quantities of wild-type and mutant COL8A2 constructs and sgRNA/Cas9 constructs and allele specificity was confirmed at the mRNA level by pyrosequencing and qPCR.

Thus, the L450W PAM-specific sgRNA was revealed to be an efficient genome editing tool, with the potential for developing a targeted therapy for FECD that will be applicable to other diseases caused by dominant-negative or gain-of-function mutations.

P24. Genetic insights into the population structure of the Sherpa and neighbouring Nepalese populations

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Nepal, located on the southern-slope of the Himalayan arc, has a complex demographic history and is home to 125 recognised ethnic groups. The Sherpa, who reside in the mountainous eastern region of Nepal, are believed to have migrated from Tibet 400-600 years ago. We set out to shed light on the population structure of eastern Nepal, in particular the Sherpa.

We established a cohort of 118 Sherpa from multiple high-altitude villages in the Khumbu region of eastern Nepal. We identified seven ethnic groups of interest from the Nepalese census that represent approximately 50 % of the total Nepalese population; Chettri, Rai, Magar, Tamang, Newar, Nepali and Aryan. We included 82 individuals from these ethnic groups, who were resident in regions in close proximity to the Sherpa.

We also included genotype-data to represent the greater Himalayan region which included individuals from the Pamir mountain-range, India, Pakistan and China. Via the analysis of dense genotype data, we investigated genetic distance, admixture and levels of homozygosity within and between populations.

Our results suggest the Nepalese are highly-admixed population with ancestry primarily from north of the Himalaya but with some geneflow from the south. We confirmed the presence of an ancestral component that appears specific to high-altitude populations of the Himalaya. This is enriched in the Sherpa, particularly in individuals from the Thame village in Khumbu. Patterns of homozygosity observed in the Sherpa and Nepalese are consistent with consanguinity and are likely to be a result of population isolation.

P25. The development and testing of a custom gene panel to aid clinical diagnosis in an adult epilepsy clinic

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Around 40,000 people in Ireland are living with epilepsy and

approximately 30% of patients are refractory to treatment. A clear understanding of the underlying cause offers the potential to improve treatment for refractory epilepsy. Further, a fast, accurate diagnosis reduces the diagnostic odyssey and associated expensive testing.

Next-generation sequencing can give complete ascertainment of genetic variation across the genome, or focused ascertainment within the coding regions of a subset of genes. This enables clinicians to identify known pathogenic mutations or novel candidate mutations that may cause a patient's epilepsy. Recent studies have illustrated the relatively high diagnostic yields for gene-panel and exome sequencing in epileptic encephalopathies, with yields in the region of 20-30%. In this study we set out to develop a gene panel for epilepsy, and test the diagnostic yield in a refractory, adult patient cohort.

We used Agilent SureDesign to design a custom gene panel containing over 400 genes linked to epilepsy and epileptic encephalopathies from published literature and a review of similar commercially available panels. We selected for sequencing a subgroup of patients (n=30) with a suspected genetic cause to their epilepsy. DNA samples were be enriched using the SureSelect QXT protocol before sequencing on an Illumina MiSeq. Alignment and variant calling was performed using Burrows-Wheel Aligner and SureCall software. We utilized databases such as OMIM, ClinVar and available literature to arrive at a set of candidate variants.

Results from this study will inform on the effective integration of next-generation sequencing to adult epilepsy clinics in Ireland.

P26. 5-Hydroxymethylation marks a class of neuronal gene regulated by intragenic methylcytosine levels

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We recently identified a class of neuronal gene inheriting high levels of intragenic methylation from the mother and maintaining this through later development. We show here that these genes are implicated in basic neuronal functions such as post-synaptic signalling, rather than neuronal development and inherit high levels of 5mC, but not 5hmC, from the mother. 5mC is distributed across the gene body and appears to facilitate transcription, as transcription is reduced in DNA methyltransferase I (Dnmt1) knockout embryonic stem cells as well as in fibroblasts treated with a methyltransferase inhibitor. However in adult brain, transcription is more closely associated with a gain in 5hmC, which occurs without a measurable loss of 5mC. These findings add to growing evidence that there may be a role for 5mC in promoting transcription as well as its classical role in gene silencing. Further interrogation of the mechanisms behind the persistence of gametic marks could potentially lead to insights into neurological disorders.

P27. The MTHFR A667C polymorphism and its association with reduced fertility

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The MTHFR gene located at 1p36.3, encodes the folate dependent enzyme 5,10-methylenetetrahydrofolate reductase, necessary for homocysteine metabolism and paramount in the creation of active vitamin B9. Defects in the gene have been associated with a plethora of pathological states such asCVD, congenital defects, reduced pregnancy and cancer.

The C677T polymorphism has been associated with various anencephalies, chiefly neural tube defects. During embryology NTD's are a known risk factor for late stage spontaneous abortion leading to reduced fertility. To assess the risk of MTHFR derived infertility we assessed 466 women attending a fertility clinic for the MTHFR polymorphism using ARMS PCR.

We found 248 (61%) of patients carried the C677T polymorphism while 181 (39%) did not carry the mutation. Of the 248 which were found to carry the polymorphism 203 were heterozygous for the mutation while 45 were homozygous. This data highlights the necessity for MTHF genetic testing. As described above, variations in the MTHFR gene may increase the risk of neural tube defects by changing the ability of methylenetetrahydrofolate reductase to process folate. Once MTHFR status is known doctors can then supplement folate more reliably.

P28. Differential responses of clinically important gene classes to transient loss of DNA methylation in human differentiated cells

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Background: Methylation of DNA sequences at promoters, CpG islands and other elements plays a vital role in regulating gene activity. In human, loss of methylation is known to play a causative role in imprinting disorders and in inappropriate germline gene expression in cancers. While in mouse, loss of function mutants have given great insight into the targets of methylation, functional studies in human have been largely limited to cancer cells and more recently stem cells, not normal adult cells. Methods: Stable knockdowns of the maintenance methyltransferase DNMT1 were generated in normosomic hTERT-immortalised adult fibroblasts. Genome-wide methylation levels were assayed using the Illumina 450K bead array. Results were analysed using RnBeads and Galaxy. Locus-specific methylation was verified using pyrosequencing and clonal analysis. Validation was achieved using transient siRNA. Results: Loss of function was poorly tolerated and all clonally-expanded cell lines had spontaneously restored DNMT1 levels by silencing of the shRNA. Evidence for a genome-wide methylation erasure event followed by a wave of remethylation could be clearly traced. Gene bodies and the shores of CpG islands showed the clearest loss of methylation overall. While most CpG islands are normally unmethylated and so unaffected, both imprints and germline genes fall into the rarer category of normally methylated islands: of these two, lasting loss of methylation was much more common among imprints than germline genes. Conclusions: 1: transient loss of methylation is poorly tolerated; 2: a robust mechanism for remethylation exists even in adult cells; 3: aberrant remethylation is frequent on recovery and 4: Imprints are particularly sensitive.

P29. Whole exome sequence analysis in a multigeneration Northern Irish family with pterygium

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Pterygium is a wing-shaped fibrovascular lesion of the ocular surface that leads to blindness in severe cases; it is reported as a premalignant condition with the potential to progress to ocular surface squamous neoplasia. Epidemiological studies show a correlation between pterygium and prolonged and intense exposure to ultraviolet (UV) light. In certain families however a much higher susceptibility to development of pterygium has been observed, suggesting a genetic cause.

Whole exome sequencing (WES) was performed to determine a causative mutation in five affected individuals and one unaffected sibling from a Northern Irish family (6 affected and 18 unaffected) with pterygium in multiple generations, in whom UV-B exposure was documented to be much lower than at equatorial latitudes.

Variant alleles identified by WES were screened with an assumption of autosomal dominant inheritance. The number of candidates genes were reduced, using different filters (confidence, common variants, predicted deleterious and genetic screening), to five, and then to one biologically plausible candidate through literature analysis. Cosegregation of the variant allele with pterygium in the family was confirmed by PCR and restriction enzyme analysis.

Expression of this gene in pterygium tissue samples, from both low UV-exposure (Northern European) and high UV-exposure (South American) populations was assessed. In vitro functional MTT and scratch assays demonstrated increased proliferation in cells transfected with the mutant compared to wild-type gene.

This study shed lights on the possible genetic mechanisms underlying pterygium formation, suggesting a role for the identified candidate gene in the general mechanism of UV-induced pterygium.

P30. Investigating the role of polymorphism rs2910164 in mir146a in cancer predisposition

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Micro(mi)RNAs are non-coding RNA molecules that bind with cis-regulatory regions in target messenger(m)RNA to exert post-transcriptional effects on gene expression, influencing a host of physiological and pathological processes. Polymorphisms in genes encoding miRNAs, or in miRNA-mRNA binding sites have been associated with cancer risk. MiR146a has a role in inflammation and is postulated to be a tumor suppressor miRNA.

The aim of this study was to investigate the frequency and impact of polymorphism rs2910164 in HSA-pre-mir146a in a cohort of patients with breast and thyroid cancer compared to cancer free controls.

The study group comprised Irish patients with breast cancer and French and Irish patients with non-medullary differentiated thyroid cancer (DTC), as well as cancer free controls. DNA from study participants was genotyped using a Taqman-based platform. Data was analysed using SPSS v22 and the Online Encyclopedia for Genetic Epidemiology studies.

The study group included 1250 patients, including 637 controls, 524 breast and 179 DTC cases. The variant was detected with a minor allele frequency (MAF) of 0.18 in controls, 0.21 in breast, and 0.28 in DTC cases. The variant conferred per allele odds ratio of 1.22(1-1.5, p=0.05, X^2) for breast, and 1.7 (1.31-2.25, p<0.0001) for DTC. An allele dosage effect was observed for both cancers, with rare homozygous genotype conferring greater risk than heterozygous for both cancer types.

A common variant in pre-mir146a is associated with breast and thyroid cancer predisposition. Further work is required to fully elucidate how this finding can be made clinically useful.

P31. The Dihydrofolate reductase 19bp polymorphism is not associated with biomarkers of folate status in healthy young adults, irrespective of folic acid intake

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Background: Dihydrofolate reductase (DHFR) is essential for the conversion of folic acid to active folate needed for one-carbon metabolism. Common genetic variation within DHFR is restricted to the noncoding regions and previous studies have focused on a 19 bp deletion/insertion polymorphism (rs70991108) within intron 1. Reports of an association between this polymorphism and blood folate biomarker concentrations are conflicting.

Objective: We aimed to evaluate whether the DHFR 19bp deletion/insertion polymorphism affects circulating folate biomarkers in the largest cohort to address this question to date.

Methods: Young healthy Irish individuals (n= 2,507) between 19 to 36 years old were recruited between February 2003 and 2004. Folic acid intake from supplements and fortified foods was assessed using a customized food intake questionnaire. Concentrations of serum folate and vitamin B-12, red blood cell (RBC) folate and plasma total homocysteine (tHcy) concentration were measured. Data were analysed using linear regression models.

Results: Folic acid intake was positively associated with serum (P <0.0001) and RBC folate concentration (P = 0.0005) and was inversely associated with plasma tHcy (P = 0.001) as expected. The DHFR 19 bp polymorphism was not significantly associated with either serum (P = 0.82) or RBC folate (P = 0.21), or plasma tHcy (P = 0.20), even in those within the highest quintile of folic acid intake (>326μg folic acid/day; P = 0.96). A non-significant trend

towards lower RBC folate by genotype (P = 0.09) was observed in the lowest folic acid intake quintile (0 – 51 μg/day).

Conclusion: In this cohort of young healthy individuals the DHFR 19bp deletion allele does not significantly affect circulating folate status, irrespective of folic acid intake. Our data rule out a strong functional effect of this polymorphism on blood folate concentrations.

P32. Mapping Human Ancestry On The New York Subway System

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In recent years the concept of analysing large amounts of data as part of the development of 'Smart Cities' has emerged. In the Pathomap project, led by Dr. Christopher Mason from Weill Cornell Medical College and with UCD ACoRD participation, DNA was sequenced from thousands of samples taken from surfaces in the New York City Subway System. The purpose being to catalogue the microbiome of the subway for the first time and monitor changes to better understand the spread of pathogens.

Human DNA was also sequenced; using the ancestry analysis software AncestryMapper and Admixture, we examined how the genetic profile of stations matched that of the ethnic and racial demographics of the area as per the 2010 census.

Hispanic and African American populations being highly admixed presented interesting challenges. Since the samples were derived from multiple individuals, the results presented many interpretations; for example, did the result indicate an area that was 20% black and 80% white or 100% Puerto Rican. Should one assume an average of ~14% European ancestry for African Americans? Is that nationally-average number appropriate for New York? We found areas with high levels of consistency, particularly ones with homogeneous populations and others where the method did not seem to match the area, few were completely against the local demographics.

In the course of this we built an automated pipeline to work through the hundreds of samples, uploading this to Curoverse, a web infrastruture that hosts open-source bioinformatic and genomic software as part of the Arvados project.

